COLOR CHANGES IN FUNDULUS AFTER HYPOPHYSECTOMY

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The pituitary gland is of primary importance in amphibian color change (Hogben, 1924) and its removal from the dogfish produces effects which are similar to those observed in the hypophysectomized frog (Lundstrom and Bard, 1932). In teleosts, however, evidence for any influence of this gland on color change is inconclusive. Certainly in *Fundulus* the activities of the melanophores, and probably of the xanthophores as well (Fries, 1931), are controlled mainly by the nervous system. Nevertheless, recent experiments have shown that other factors, probably humoral in nature, exert some control over the denervated melanophores (Smith, 1931; Parker, 1932) and xanthophores (Fries, 1931), enabling them to respond in an appropriate manner to changes in background though much more slowly than do those pigment cells which remain connected with the nervous system. As Smith points out, these factors may play some part in the activities of innervated pigment cells as well, "probably—furthering the action of the nervous system when the animal remains on the same background for a relatively long period." The nature of the hormone which produces these effects, however, is in doubt. Parker believes that neurohumoral substances are responsible for the changes in tint of denervated areas in *Fundulus*. Smith states that "while in Phoxinus at least humoral factors exert an influence upon the behavior of the melanophores it is at the present moment impossible to enlarge upon this statement," but he suggests the possibility of adrenalin and pituitrin playing a part. Certainly extracts of the posterior lobe of the pituitary do produce definite changes in the pigment cells of both *Fundulus* and *Phoxinus*. Spaeth (1918) found that pituitrin produced a concentration of melanophore pigment when isolated scales of *Fundulus* were immersed in it, and Wyman (1924) obtained similar results with injections of this drug. Hewer (1926) also described a concentration of melanophore pigment and in addition a dispersion of xanthophore pigment following the injection of pituitrin into a minnow, although he is of the opinion that the pituitary gland plays no part in normal color change since the amount of pituitrin necessary to bring about these changes is, he believes, too large to be produced by the animal itself. In *Phoxinus* the effects of injections of pituitary extracts are apparently different from those observed in *Fundulus*. Abolin (1925) obtained in this fish a dispersion of melanophore, xanthophore, and erythrophore pigments following an injection of infundin and he believed the injection acted in the same way as does the normal reaction to a black background. Similarly, as a result of an injection of infundin, Giersburg (1930) observed a definite dispersion of pigment granules or "expansion" of the melanophores on the sides of *Phoxinus*, together with a pronounced "expansion" of the xanthophores and erythrophores, although he did obtain a "contraction" of the melanophores on the back of this fish. He suggests that the xanthophores and erythrophores and possibly a few melanophores (*e.g.* those on the distal parts of the fins) are controlled by humoral factors, "expansion" of these cells being a result of pituitary activity.

Recently Zondek and Krohn (1932, *a* and *b*) have isolated a substance which they call *intermedin*, from the intermediate lobe of the pituitary of various animals including *Phoxinus*, which, when injected into this fish, produces an "expansion" of the melanophores, xanthophores, and erythrophores. These authors claim that this substance is the only one which will produce this expansion of the erythrophores and that duplication of the red coloration which this fish exhibits during the breeding season can be obtained by injecting a suitable amount of intermedin.

It would appear, then, that humoral factors probably play some part in the color changes of teleosts. Moreover, since the injection of pituitary extracts produces definite changes in color in these fishes, the pituitary gland may be suspected of taking some part in this humoral influence on color change, particularly since this gland is of such importance in color changes of amphibians and its removal has such a pronounced effect on the color pattern of the dogfish. In order to determine what rôle, if any, is played by the pituitary gland in color changes in *Fundulus* the following experiments were carried out.

The hypophysis was removed from a number of killifish (*Fundulus heteroclitus*) in the following way. Each fish was fastened on its back by two strips of wet cloth that were passed across its body and secured to a piece of cork with thumb tacks. A V-shaped cut through the branchiostegal membrane was made with the base of the V at the tip of the tongue. The tongue was then pulled ventrally through the incision which was extended dorso-laterally far enough to expose the region of the hypophysis in the roof of the oral cavity. Usually the hypophysis itself could be seen through the mucous membrane and bone. The mucous membrane was now cut longitudinally a little to one side of the mid-line (to avoid a median artery) and the tissues loosened from the bone. At first a dental burr was used to penetrate the bone ventral to the hypophysis but this approach left the field so clouded with bone dust that it was difficult to be certain when the hypophysis was completely removed. To avoid this in the majority of cases the bone was cut through with iridectomy scissors and a small window removed, thus exposing the hypophysis which could then be drawn out with forceps or with a fine pipette. The *circulus cephalicus* was frequently cut on one side, but the animals recovered from these operations about as readily as when none of the main arteries had been cut. After the operation no attempt was made to close the incision in the floor of the mouth. The animals were merely kept in N/10 sodium chloride for from 24 to 48 hours and then removed to tap water. In all cases the individuals were examined after death to determine whether or not the hypophysis had been completely removed. Those cases where part of the gland was left behind served as controls. Additional control animals were obtained by performing a number of dummy operations in which the hypophysis was exposed but left intact.

Animals hypophysectomized in this way lived for several weeks, during which time their reactions to background changes were examined. As usual a number failed to react, a common experience even with unoperated animals. The majority of both the hypophysectomized and control animals, however, reacted exactly as do normal animals when placed over white, black, or yellow backgrounds, becoming light over white, dark over black, and a decided yellow color over the yellow background. Microscopic examination of the tail fins of these animals showed that, as usual, the melanophores were contracted over white and yellow backgrounds and expanded over black, while the xanthophores were contracted over white and expanded over yellow. Many of the animals became lighter in color a few days after the operation due to degeneration of some of the melanophores, as could be seen when a scale or the tail fin was observed under the microscope. But here again no difference could be detected between hypophysectomized and control animals, hence the bleaching of these animals could not have been due to the absence of the hypophysis.

Parker and Lanchner (1922) have shown that when a *Fundulus* is put in total darkness it becomes light due to contraction of its melanophores. This reaction was also tested. When a light-proof box was placed over the aquarium in which a hypophysectomized animal was swimming, the removal of this hood an hour or so later showed that the fish had become definitely lighter in color, again behaving like a normal animal.

In order to determine whether or not the pituitary body is concerned with the slow changes in tint of denervated areas which Smith (1931) and Parker (1932) have observed, the following experiments were performed. A transverse cut was made in the base of the tail of six animals following the technique described by Fries (1931). A black area extending distal to the cut gave evidence that the nerves running to the melanophores in this area had been severed. The hypophyses were then removed from three of these animals, the other three serving as controls. When kept continuously over a white background it was found that the denervated melanophores of the tail contracted at approximately the same time in both hypophysectomized and control animals, about three days being required to complete the change in both cases. Similar results were obtained for denervated areas of the trunk. Although the reaction here was not as definite as that in the tail, nevertheless black denervated areas of the trunk did become smaller after several days over a white background in hypophysectomized as well as in control animals. Obviously removal of the hypophysis does not prevent denervated areas from changing in tint when the fish are transferred from one background to another.

It is apparent from these experiments that the removal of the hypophysis from *Fundulus* does not affect the ordinary reactions which it exhibits to changes in background or to total darkness. But since commercial posterior lobe extracts do cause the melanophores of this fish to contract (Spaeth, 1918; Wyman, 1924), the question naturally arises as to whether or not the pituitary body of *Fundulus* contains any of the melanophore principle. The pituitary bodies of other fish have been shown to contain this principle. Hogben and Winton (1922) found that extracts of cod pituitary produced the usual effects when injected into frogs, and Zondek and Krohn (1932*b*) have shown that "intermedin" can be extracted from the pituitary of *Phoxinus*. The following experiment shows that the hypophysis of *Fundulus* also contains the melanophore principle. The pituitary, forebrain, medulla, and a small piece of trunk muscle were removed from a *Fundulus* of average size (6 to 8 centimeters in length) and separate extracts were made of each by grinding them in 0.05 cc. of N/10 sodium chloride solution. As test objects a number of scales from another fish were mounted in hanging drops of the same medium in which, as usual, the xanthophores contracted and the melanophores expanded. When the fluid on one of these scales was exchanged for the brain or muscle extracts no change was observed in the pigment cells In fact, when scales with contracted melanophores and expanded xanthophores were mounted in these brain extracts, the melanophores would expand and the xanthophores contract, the brain extract thus having the same effect that ordinary sodium chloride solutions would have. But when a scale preparation was changed to the pituitary extract, the xanthophores always expanded, while the melanophores contracted. Obviously these extracts of the pituitary body were crude and probably only a small proportion of the active principle or principles of the posterior lobe could be removed in this way (Kamm et al., 1928). As a matter of fact, the reaction of the pigment cells was slower than that produced by N/10 KCl, for example, as several minutes were required for the maximum effect to be reached and even then the melanophores were not always contracted to a punctate condition. Moreover, the effect of the pituitary extract lasted for only about 45–60 minutes, after which the melanophores slowly reëxpanded, and although they could be made to contract again by adding the extract a second time, this second contraction was rarely as complete as the first. Nevertheless, these experiments show that the pituitary body of *Fundulus* does contain a substance which will produce an expansion of the xanthophores and a contraction of melanophores in isolated scale preparations. How this fact is to be reconciled with the results described above, namely that the removal of this gland has no apparent effect on subsequent color changes of the animal, is not evident from these experiments. It is possible, as Hewer (1926) suggests, that any melanophore principle formed by the hypophysis is liberated in quantities too small to have any effect on the pigment cells. However this may be, it is clear from the experiments first described that the removal of the hypophysis from a *Fundulus* does not affect the ability of that animal to change its color in apparently the same manner as does a normal animal.

Summary

When the hypophysis of a *Fundulus* is ground up in 0.05 cc. N/10 NaCl and a scale from this fish is mounted in such an extract, the xanthophores "expand" while the melanophores "contract," although brain and muscle extracts prepared in the same way have no effect on the pigment cells. The hypophysis of *Fundulus* does contain the melanophore principle. In spite of this fact, when the hypophysis is removed from *Fundulus*, the animal still responds to changes in background and to total darkness in the usual manner. Moreover, the slow change in tint of denervated areas occurs as readily in hypophysectomized as in normal animals. In other words, the removal of the

hypophysis has no apparent effect on the pigmentary responses which *Fundulus* may exhibit.

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